representation to the MEC. Regardless of the site of origin of the spatial signal, our results demonstrate a fundamental distinction between the functional correlates of the two major streams of input into the hippocampus from the neocortex. Superficial layers in the MEC contain exquisitely tuned "place cells," which may arise from a grid-like representation of space (fig. S6) (*16*). In contrast, there were few robust place cells in the superficial layers of the LEC under the present conditions of unstructured foraging in an environment with few spatial landmarks. Perhaps cells from the dorsolateral band of the LEC display spatial firing under other conditions (e.g., in a visually complex environment or in a more structured behavioral task). Alternatively, because CA1 neurons respond to individual items or discrete stimuli in conjunction with spatial location (*3*–*5*), it is possible that the LEC stream carries this nonspatial information from the perirhinal cortex into the hippocampus, where it is combined with spatial information from the MEC stream to create conjunctive object-place (or event-place) representations in the hippocampus proper (*28*, *29*). Consistent with this notion of parallel input streams, perirhinal cortex lesions disrupt exploratory behavior based on novel configurations of objects, whereas postrhinal cortex lesions disrupt exploratory behavior based on novel configurations of an object and a spatial context (*30*). This process may be a rodent analog of a dissociation in humans between item and source memory localized to the perirhinal and parahippocampal cortices, respectively (*31*).

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23. Because there were no differences in the spatial information scores between the deep and superficial layers of either the parasubiculum or perirhinal cortex, recordings from these layers were combined for each area. Comparing the information scores of CA1, the superficial MEC, and the superficial LEC, with only the superficial cells of the parasubiculum or perirhinal cortex, yielded no change in the results.
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32. We thank M. Witter for reviewing recording site locations and M. Shapiro and J. Ferbinteanu for helpful comments on the manuscript. Supported by the Texas Higher Education Coordinating Board Advanced Research Program 011618-0180-1999 and by grants R01 NS039456 and K02 MH63297 from the Public Health Service.

Supporting Online Material

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31 January 2005; accepted 14 April 2005
10.1126/science.1110449

Early Asymmetry of Gene Transcription in Embryonic Human Left and Right Cerebral Cortex

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The human left and right cerebral hemispheres are anatomically and functionally asymmetric. To test whether human cortical asymmetry has a molecular basis, we studied gene expression levels between the left and right embryonic hemispheres using serial analysis of gene expression (SAGE). We identified and verified 27 differentially expressed genes, which suggests that human cortical asymmetry is accompanied by early, marked transcriptional asymmetries. *LMO4* is consistently more highly expressed in the right perisylvian human cerebral cortex than in the left and is essential for cortical development in mice, suggesting that human left-right specialization reflects asymmetric cortical development at early stages.

One of the most remarkable aspects of the human cerebral cortex is that the two hemispheres are specialized for distinct cognitive and behavioral functions. Whereas the right cerebral cortex regulates movement of the left side of the body and vice versa, ~90% of the human population is naturally more skilled with the right hand than with the left (*1*). This motor asymmetry is strongly correlated with language dominance: Language function is predominantly localized to a distributed network in the left perisylvian cortex in 97% of right-handers and ~60% of left-handers (*2*, *3*). Functional asymmetries exist in mathematical ability and in spatial and facial recognition as well. These functional asymmetries have been related to anatomical asymmetries of the cortex that are somewhat more subtle (*2*, *4*). For example, the posterior end of the sylvian fissure is higher in the right hemisphere than in the left (*5*). The planum temporale, a region in the posterior portion of the superior temporal sulcus in which Wernike's area resides, is larger in the left hemisphere than in the right in more than 65% of examined adult and 56 to 79% of examined fetus and infant brains, so the anatomical asymmetries are less marked than

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the functional ones (*6*, *7*). Although genetic factors connecting cerebral asymmetry and functional dominance have been supported (*8*), no molecular correlate of cerebral asymmetry has been identified.

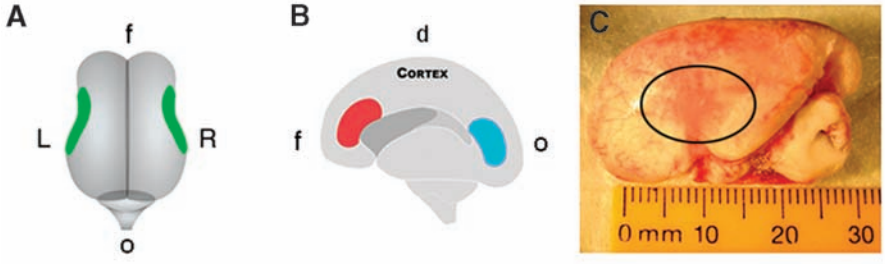
We directly tested the hypothesis that left-right cortical asymmetry in humans results from differential gene expression at early embryonic stages, long before the onset of organized cerebral cortical function. By applying serial analysis of gene expression (SAGE), we measured gene expression levels between the left and right hemispheres in early (12- to 14-week-old) fetal human brains, during periods of neuronal proliferation and migration, and later (at 19 weeks), after these processes are largely completed (*9*). Brain tissues were first dissected from matching perisylvian regions in two hemispheres (Fig. 1, A to C). The cortex was then separated at the midline. On the medial side of the hemisphere, tissues were also dissected from the ventricular zone in the frontal and occipital regions (Fig. 1B). Total RNA was isolated, and 14 SAGE libraries were generated (Fig. 1D). To detect genes with differential expression levels, we compared the tag frequency for each gene between two SAGE libraries generated from the frontal, perisylvian, and occipital regions in the left-right hemispheres. To verify the statistical significance of differences in each comparison, we performed a Monte Carlo test and verified this using the χ test. Using the χ-squared distribution with one degree of freedom and confidence levels (P), we sorted genes within each comparison (e.g., left-right). A higher χ value indicates a greater statistically significant difference.

In all, 49 differentially expressed genes were identified by SAGE with $P > 99\%$ (χ value > 6.63) between the left-right perisylvian regions of a 12-week-old embryonic human cortex. Among them, 21 genes were highly expressed in the left region, whereas 28 genes were highly expressed in the right (Fig. 1E). Moreover, 68 genes were identified with $P > 99\%$ between the left-right perisylvian regions of a 14-week-old cortex (Fig. 1E). By combining analyses, we generated a list of statistically differentially expressed genes between the left-right hemispheres in the perisylvian regions (Fig. 1E and tables S1 to S5) and frontal and occipital regions (tables S9 to S12) of human embryonic brains at 12, 14, and 19 weeks. Differential gene expression levels detected by SAGE suggested an early transcriptional asymmetry between the left-right hemispheres in human embryonic brains.

One of the genes reproducibly asymmetrically expressed was the transcription factor *Lim Domain Only 4* (*LMO4*). Using SAGE analysis, we found that the human *LMO4* is more highly expressed in the perisylvian regions of the right hemisphere than in the left at both 12 and 14 weeks (Fig. 2A). In contrast, *LMO4* expression levels did not show significant differences between the left and right perisylvian regions at 19 weeks (Fig. 2A). We then quantified *LMO4* expression levels using real-time SYBR (Applied Biosystems)–green reverse transcription–polymerase chain reaction (RT-PCR) and confirmed higher *LMO4* expression in the right perisylvian regions than in the left of embryonic 12- and 14-week-old but not 19-week-old brains, using the same RNA samples for SAGE analysis (Fig. 2B). Moreover, we confirmed higher levels of *LMO4* expression in the right perisylvian region versus the left in second 12-week-old and 14-week-old brains and observed modest differences in two 16-week-old brains and one 17-week-old brain (Fig. 2B).

We next performed nonradioactive in situ hybridization on human embryonic brains and noted right-left differences in the extent of *LMO4* expression at 12 weeks. We serially sectioned the cortex in the frontal plane and performed in situ hybridization on at least 54 sections from this series, covering most of the frontal to occipital extent of the cerebral cortex (Fig. 2C). Consistent with the early expression of *Lmo4* in mice (see below), *LMO4* in this 12-week-old human brain was expressed in the ventral lateral cortical plate in a patchy fashion (Fig. 2, D to F). *LMO4* was also expressed highly and symmetrically in noncortical telencephalic structures, notably the putamen (Fig. 2F). We analyzed the medial-lateral extent of *LMO4* expression in the cortical plate in relation to the lateral border of the basal ganglia, defined by connecting the corticostriatal sulcus and the lateral border of the putamen (Fig. 2F). In this brain, *LMO4* expression was observed further dorsolateral in the cortical plate in the right hemisphere than the left, particularly in sections near the future perisylvian region (Fig. 2F).

We then confirmed asymmetric *LMO4* expression in several additional human fetal brains, focusing in the perisylvian region, using [^{35}S]-labeled radioactive in situ hybridization. At 14 weeks, *LMO4* was highly expressed in the cortical plate around the entire perimeter of the cortex. Although levels of *LMO4* expression in the right hemisphere



D

RNA Samples				SAGE Tags					
Code	Embryonic Stages	Sex	Application	Frontal Region		Perisylvian Region		Occipital Region	
				L	R	L	R	L	R
#1438	12.6 weeks	M	Making SAGE libraries	55,979	57,477	61,671	60,193	61,679	63,000
#1392	12.5 weeks	M	Verify SAGE data						
#1341	14 weeks	F	Making SAGE libraries	56,000	61,930	54,237	56,595	64,986	62,000
#1316	14 weeks	M	Verify SAGE data						
#1335	19.3 weeks	M	Making SAGE libraries			62,575	61,868		

E

Embryonic stages	12 weeks				14 weeks				19 weeks			
Confidence level	p>99%		95%<p<99%		p>99%		95%<p<99%		p>99%		95%<p<99%	
Comparison	L>R	L<R	L>R	L<R	L>R	L<R	L>R	L<R	L>R	L<R	L>R	L<R
Frontal Region	25	26	217	172	28	17	145	152	n/d	n/d	n/d	n/d
Perisylvian Region	21	28	189	180	38	30	194	117	14	14	166	196
Occipital Region	37	13	201	174	48	29	189	218	n/d	n/d	n/d	n/d

Fig. 1. Dissection of human embryonic brain tissues and generation of human SAGE libraries. (**A**) A top view of a 14-week-old human embryonic brain. Tissues were dissected from perisylvian regions in the left (L) and right (R) hemispheres. (**B**) A side view of a 14-week-old human embryonic right hemisphere. Tissues in the frontal (f, red) and occipital (o, blue) ventricular regions containing dividing cells were dissected. The dorsal cortex (d) is on the top. (**C**) The left side view of a 14-week-old human embryonic brain. The perisylvian region is circled. (**D**) Summary of human brain SAGE libraries. The male (M) and female (F) brains are listed. At least 55,000 tags were sequenced in each library. (**E**) Summary of differentially expressed genes detected by SAGE analysis between the left-right hemispheres. Numbers of genes that are highly expressed in the left (L > R) or right (L < R) hemisphere are listed with confidence levels $P > 99\%$ and $95\% < P < 99\%$. n/d, not detected.

were comparable to those in the left in broad areas of dorsomedial neocortex, paralleling the fact that there are no known anatomical asymmetries of these medial areas, it was consistently more highly expressed over broad areas of the right perisylvian cortex than the left (Fig. 2, G to J). At 16 weeks, asymmetric cortical expression was still observed, although it was diminished relative to earlier time points, consistent with the real-time RT-PCR analysis (Fig. 2, B, K, and L). Marked asymmetries in *LMO4* expression were seen in the perisylvian region of a human 17-week-old cortex studied by nonradioactive in situ hybridization (fig. S1) and were even more apparent than in the RT-PCR results (Fig. 2B). In a 19-week-old brain, consistent with RT-PCR results, left-right differences in *LMO4* expression were not obvious (fig. S1). Overall, the in situ hybridization analysis mirrored the SAGE and RT-PCR analysis, with clearer but variable asymmetries in expression among individuals at earlier stages and no clear asymmetry at the latest stage examined (19 weeks).

To better understand the dynamic change in *Lmo4* expression during cortical development, we analyzed *Lmo4* expression in mouse brains. Similar to its expression in the 12-week-old human embryonic brain, *Lmo4* was weakly expressed in the ventral cortex in the embryonic day 11.5 (E11.5) mouse brain, and its expression increased during development (Fig. 3A). *Lmo4* expression boundaries were fairly sharp at postnatal day 1 (P1) with expression being high in the anterior and posterior portions of the cortex, but with a large zone of nonexpression that overlapped the presumptive parietal cortex in between (Fig. 3D). However, this nonexpression zone disappeared at P17 (Fig. 3G). In coronal sections of E15.5 and P5 mouse cortices, *Lmo4* was expressed in the medial and lateral cortical areas as well (Fig. 3, B and E). *Lmo4* in the mouse showed apparent asymmetries in the cortical area in which it was highly expressed, and the expression pattern was quite dynamic (Fig. 3, A to G).

Because the levels of coronal sections may affect in situ hybridization signal, we mapped the *Lmo4* expression on serial sagittal sections to provide an accurate gene expression pattern (Fig. 3H). We divided the cortical expression of *Lmo4* in P1 brain into three regions: anterior (expression I), intermediate (nonexpression), and posterior (expression II) (Fig. 3I). The ratios of the length of each *Lmo4* expression region versus the full length of the cortex were calculated from the medial to lateral cortical regions and analyzed with histograms (Fig. 3, J and K). The *Lmo4* expression areas in the anterior were smaller in the left hemisphere than in the right in six tested brains (shown by one representative brain in Fig. 3J), but larger in the left than the right in four tested brains (shown by one representative brain in Fig. 3K). Correspondingly, the *Lmo4* nonexpression areas (intermediate) were larger in the left hemisphere than in the right in the same six tested brains (Fig. 3J) but smaller in the same four tested brains (Fig. 3K). However, we did not detect asymmetric *Lmo4* expression in the posterior cortex between the left-right hemispheres in any of the tested brains (Fig. 3, J and K). Thus, although *Lmo4* expression in mouse cortex was moderately asymmetrical in every individual brain tested so far, it was not consistently lateralized to the right or left

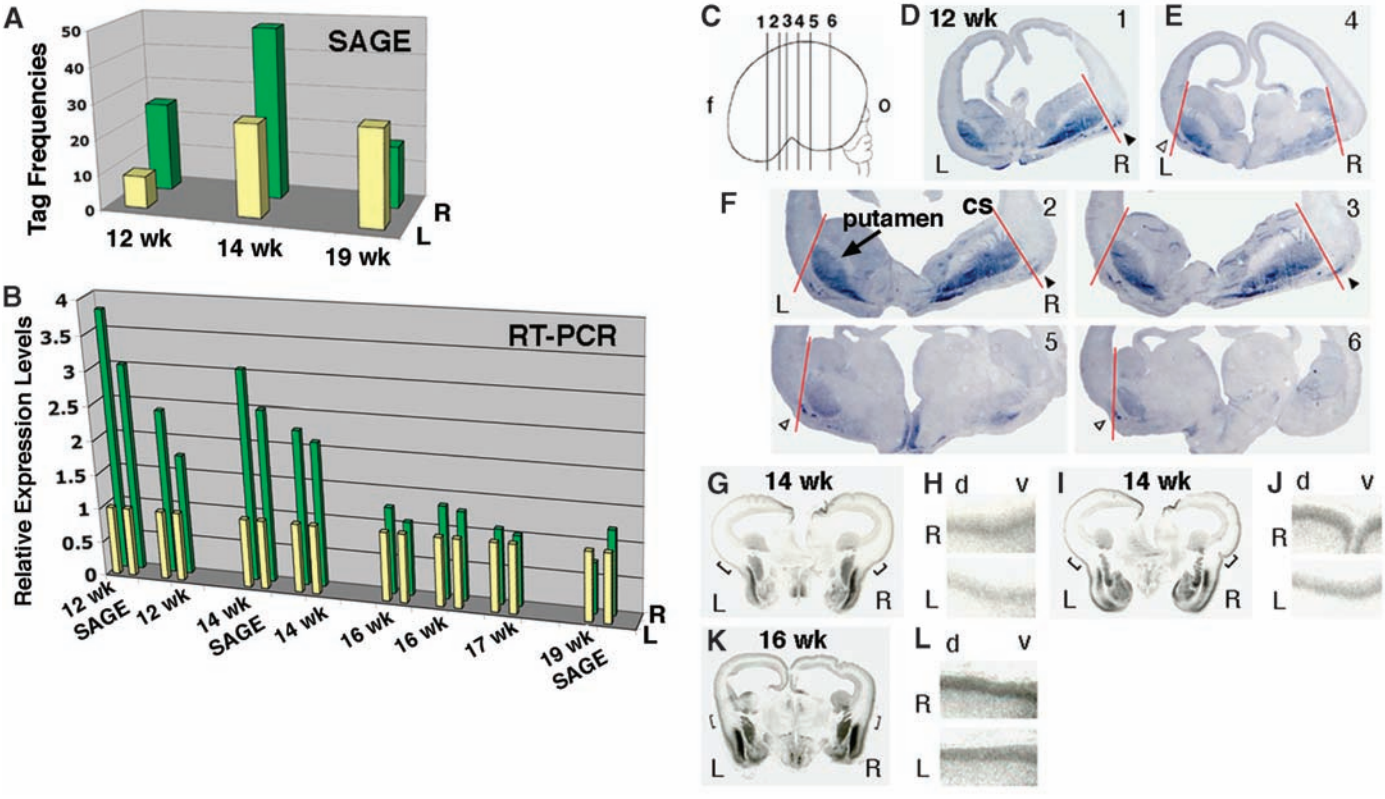


Fig. 2. Human *LMO4* was highly expressed in the right hemisphere as detected by SAGE, real-time RT-PCR, and in situ hybridization. (**A**) The human *LMO4* expression levels in the perisylvian regions measured by SAGE (tag frequencies) in 12-, 14-, and 19-week-old brains. wk, week. (**B**) The *LMO4* expression levels between the left and right hemispheres were verified by real-time RT-PCR in eight human embryonic brains (at 12 to 19 weeks). Two data points from duplicated experiments for each sample are illustrated. (**C** to **F**) *LMO4* expression in coronal sections cut from the frontal (f) to occipital (o) lobes of a human embryonic 12-week-old brain. The medial-lateral extent of *LMO4* expression in the cortical plate between the left (white arrowheads) and right (black arrowheads) hemispheres was defined by a red line connecting the corticostriatal sulcus (cs) and the lateral border of the putamen (arrow). Numbers in (D) through (F) indicate the sections shown in (C), (**G** to **L**). Human *LMO4* was more highly expressed in the cortical plates in the right hemispheres than in the left in coronal sections of [(G) to (J)] a 14-week-old brain and [(K) and (L)] a 16-week-old brain. (H), (J), and (L) show high-power views of selected areas in (G), (I), and (K), respectively. (G) and (I) illustrate two different rostral-caudal levels through the frontal lobe and presumptive perisylvian region. The dorsal (d) and ventral (v) areas of the cortex are labeled.

side. This may relate to behavioral and anatomical studies in mice, in which sensory-motor asymmetries, like paw preference, are observed in individual mice but are not biased on a population level to either the right or left hemisphere, as hand preference is in humans (*10*–*14*). The differences in mice and humans suggest the possibility that paw preference in rodents might reflect an early, perhaps stochastic, developmental asymmetry that is established perinatally, before paw usage, implying a transcriptional asymmetry that is not consistently lateralized to the left and right. Evolution of mechanisms that bias or entrain a modest and random asymmetry in lower organisms may have allowed the development of more consistent functional asymmetries in the human cortex (*15*).

To identify other differentially expressed genes between the left-right hemispheres, we focused on genes showing different expression levels measured by SAGE in the perisylvian regions of human embryonic 12-week-old brains. Using RNA samples for generating SAGE libraries, we verified 76 genes using real-time RT-PCR (tables S6 and S7) and found 39 genes (51%) showing consistent differential expression as measured by SAGE (table S6). To further test the reproducibility of verifying SAGE data, we verified expression levels of these 76 genes using a second 12-week-old brain. We found that 27 genes (36%) consistently showed differential expression (either left^{-high} or left^{-low}) in both brain samples (table S6). We also tested 17 genes that have lower χ values (<1.9) but have been implicated in cortical development and found 7 genes showing relatively significant differences of gene expression levels, although they were not detected by SAGE (table S8).

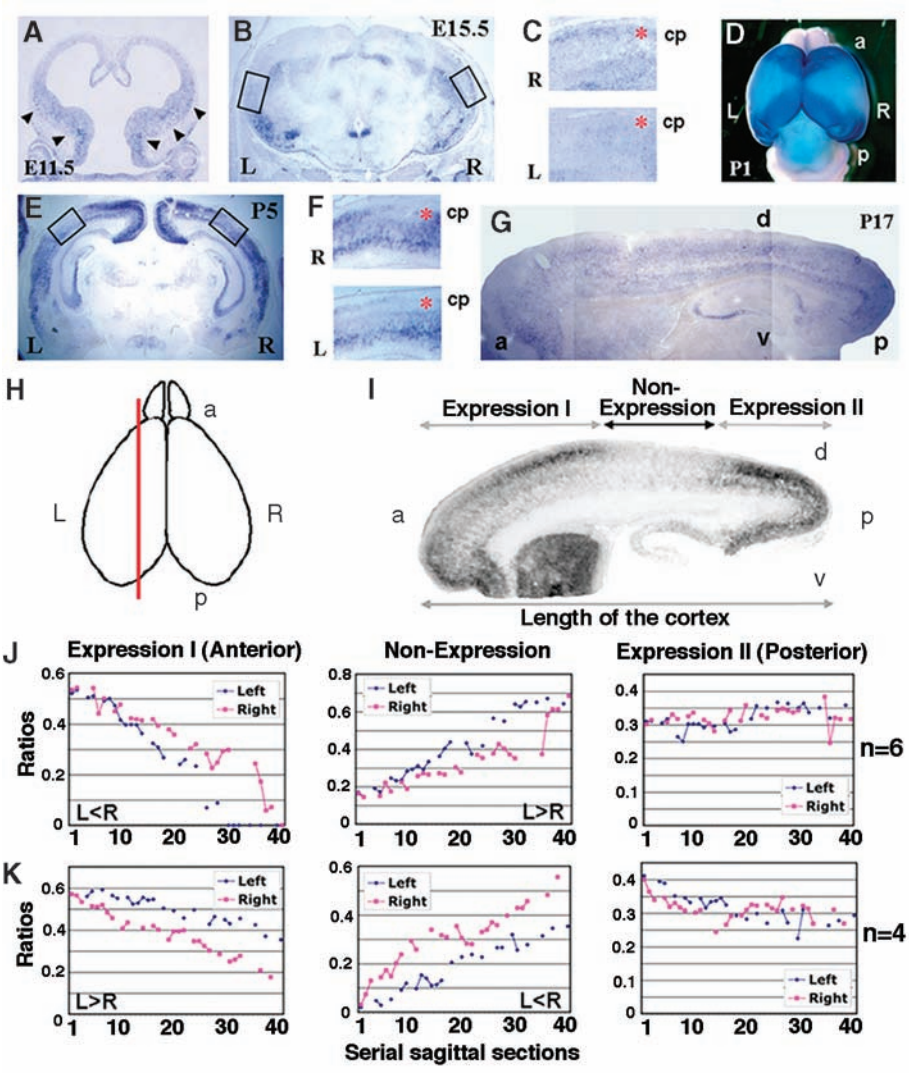


Fig. 3. The dynamic and asymmetric expression of *Lmo4* in mouse brains. (**A** to **F**) The patchy and asymmetric expression patterns of *Lmo4* are illustrated in [(A) to (C)] coronal sections of mouse brains from (A) E11.5 and (B) E15.5; (D) whole mount in situ hybridization from P1; and (E and F) coronal sections of P5 brain. (C) and (F) show high-power views of *Lmo4* expression in the cortical plates (cp) (red stars) in selected areas from (B) and (E), respectively. The dorsal cortex is on the left of (C) and (F). (**G**) *Lmo4* expression appears uniform in the cortical plate in a sagittal section of a P17 brain. (**H**) Schematic view of the forebrain from above, indicating the sagittal section shown in (I). (**I**) *Lmo4* expression was divided into the anterior (a) (Expression I), the posterior (p) (Expression II), and the nonexpression regions in sagittal sections of P1 mouse cortex. The dorsal (d) and ventral (v) areas of the cortex are labeled. (**J** and **K**) Asymmetric expression of *Lmo4* in the left-right hemispheres in representative P1 mouse cortices. The ratio of the size of each domain in the left versus the right hemisphere is plotted for serial sagittal sections. In total, six brains were similar to that in (J) and four brains to that in (K).

The left-right differences in *LMO4* expression in humans could potentially reflect either a differing topographic mapping in the two hemispheres or a difference in the tempo of cortical development, with the right hemisphere's development leading over the left. In mice, *Lmo4* expression marks anterior and posterior regions in the mouse cortex and is shifted in cortices of *Pax6* and *Fgf8* mutants, consistent with the suggestion that *Lmo4* expression at P0 to P5 reflects overall cortical topographic mapping (fig. S2). On the other hand, there is some evidence for the appearance of the several cortical sulci and gyri at earlier ages in human right hemisphere than in the left, for instance, the rolandic sulcus (which appears at 17 to 20 weeks) and the superior temporal fissure (at 23 weeks) (*2*, *7*, *16*). Thus, higher *LMO4* expression in the right than the left hemisphere could reflect the arrival of corresponding developmental stages sooner in the right than the left hemisphere. Either model, however, implies molecular events that greatly precede morphological asymmetries and provide potential insight into the mechanism of generation of asymmetry. Furthermore, the molecular events that regulate *LMO4* expression in humans may be secreted molecules, such as FGF8, and/or gradients in transcription factors in the ventricular zone, such as PAX6 and EMX2, as in mice. Indeed, some factors with potential roles in cortical development, such as *ID2* and *NEUROD6,* were asymmetric in SAGE and/or RT-PCR analyses in human embryonic 12-week-old brains (tables S6 and S8). Understanding the factors that regulate *LMO4* expression may ultimately identify earlier events in left-right brain asymmetry. On the other hand, our results generally confirm the earlier suggestion that genes previously implicated in visceral asymmetries are not detectably implicated in cerebral asymmetries (*3*, *17*). For instance, mutations that result in "situs inversus" in humans do not appear to disrupt the left-hemisphere localization of language, mathematical, and hearing abilities and handedness (*18*). Except possibly for the FGF signaling pathway, we did not detect significant differences of expression levels of genes regulating body asymmetry in the human

embryonic SAGE libraries, although asymmetries at earlier developmental stages cannot be ruled out (*3*, *19*).

Abnormalities of cerebral cortical asymmetry have been reported in a wide array of neuropsychiatric disorders, such as autism, schizophrenia, and dyslexia (*20*–*23*). The presence of early asymmetries in gene transcription in the two cerebral hemispheres thus provides potential pathways through which a number of developmental disorders may ultimately converge on the abnormal development of human cerebral asymmetry.

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24. We thank Y. F. Chen, J. Meschter, V. Petkova, and P. Grosu for technical support; S. Blackshaw, K. Polyak, and E. Fox for advice on SAGE; R. Vigorito, R. Johnson, and B. Poulos at the NIH-funded Brain and Tissue Banks; S. Dymecki for supplying *Pax6* mutant brains; and E. Meyers and G. Martin for *Fgf8* mutant brains. Supported by the McKnight Endowment Foundation, National Institute of Neurological Disorders and Stroke grant nos. RO1 R37 NS35129 (C.A.W.) and MH60233 (D.H.G.), and the James S. McDonnell Foundation (D.H.G.). T.S. is supported by an Advanced Postdoctoral Fellowship from the National Multiple Sclerosis Society and an NIH training grant from the Children's Hospital, Boston. S.H.O. and C.A.W. are Investigators of the Howard Hughes Medical Institute.

Supporting Online Material

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27 January 2005; accepted 12 April 2005
Published online 12 May 2005;
10.1126/science.1110324
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Complementary Process to Response Bias in the Centromedian Nucleus of the Thalamus

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Activity in several areas of the human brain and the monkey brain increases when a subject anticipates events associated with a reward, implicating a role for bias of decision and action. However, in real life, events do not always appear as expected, and we must choose an undesirable action. More than half of the neurons in the monkey centromedian (CM) thalamus were selectively activated when a small-reward action was required but a large-reward option was anticipated. Electrical stimulation of the CM after a large-reward action request substituted a brisk performance with a sluggish performance. These results suggest involvement of the CM in a mechanism complementary to decision and action bias.

When a large reward is expected as a result of a particular action, animals tend to choose this action more frequently than other alternatives (*1*, *2*). Animals that expect a reward carry out these activities quickly and with few errors (*3*–*5*). Studies have been undertaken to understand the neural correlates of decision-makers that assess the reward value of response options and set a "bias" to produce a particular response. Single-neuron activity reflects an expected reward and a schedule to obtain a reward in the cerebral cortex (*6*–*10*) and in the basal ganglia (*3*, *5*, *11*). Midbrain dopamine neurons transmit error of reward expectation (*12*–*14*) and motivation to action (*13*, *15*) to the striatum, limbic system, and cerebral cortex. On the other hand, the traditional models of how actions are produced may be fundamentally incomplete as the basis of goal-directed action mechanisms, because response bias is sometimes aborted when events do not occur as expected. Thus, an additional component, which plays complementary roles to response bias, seems to be required. Through both response bias and its complementary process, animals seem to be able to attain their final goals. However, the neural basis of this process has not been elucidated.

The centromedian/parafascicular (CM/PF) complex of the thalamus (*16*) has received little attention in studies of action and cognition, even though it has close connections to the basal ganglia and cerebral frontal cortex (*16*–*20*). Neurons in the monkey CM/PF complex respond to multimodal stimuli. Characteristically, the magnitude of a response is larger when the stimulus is unpredictable and contrary to expectation (*20*, *21*). This is in contrast to the basal ganglia, where signals of reward expectation are dominantly represented (*3*, *5*, *11*, *22*). Based on the complementary nature of neuronal activity between the CM/PF and the basal ganglia, it has been hypothesized that the CM/PF complements decision and action bias through the thalamostriatal projection and corticobasal ganglia loops (*21*, *23*). This study was designed to test this hypothesis, with special emphasis placed on the action-related CM neurons.

Two macaque monkeys (SJ and MA) were trained to perform GO-NOGO tasks in which visual stimuli asking for the GO or the NOGO actions appeared at an average probability of 0.5 (*24*). Performance of either the requested GO or NOGO action was rewarded by a large amount of water (+R), whereas the performance of the other action was rewarded with a small amount of water (−R) during a block of 60 to 120 trials. The action-reward association was then altered in the subsequent block (Fig. 1, A and B). Monkeys performed the large-reward GO trials more briskly than the small-reward trials. Average reaction times in the GO(+R) condition were shorter than those in the GO(−R) condition by 253 ms in monkey SJ (unpaired t test, $P < 0.0001$, 95% confidence interval (CI) 237 to 268 ms) and 89 ms in monkey MA ($P < 0.0001$, 95% CI 83 to 93 ms) (Fig. 1C). Movement times were shorter in the GO(+R) than in the GO(−R) condition by about 20 ms in both monkeys. Furthermore, the rate of error trials, such as reaction times that were too long (>3000 ms) or the initiation of incorrect actions, was higher in the small-reward trials (monkey SJ, GO trials, $P = 0.006$, 95% CI 0.34% to 1.91%; NOGO trials, $P = 0.023$, 95% CI 0.26% to 3.37%) (monkey MA, NOGO trials, $P = 0.002$, 95% CI 1.78% to 6.33%; GO trials, monkey made no error in +R or –R conditions).

The activity of 56 neurons (40 in monkey SJ and 16 in monkey MA) was recorded. The neurons showed burst discharges after un-

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